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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants:	R.B. Croteau et al.	Attorney Docket No.:	WSUR117920
Application No.:	09/934,778	Group Art Unit:	1634
Filed:	August 21, 2001	Examiner:	F.W.M. Lu
Title:	GERANYL DIPHOSPHATE SYNTHASE LARGE SUBUNIT, AND METHODS OF USE		

RESPONSE

Seattle, Washington 98101

April 6, 2005

TO THE COMMISSIONER FOR PATENTS:

In view of the arguments that follow, applicants respectfully submit that all of the pending claims are in condition for allowance. Reconsideration and favorable action are requested.

Rejection of Claims 10, 18, and 43 Under 35 U.S.C. § 112, First Paragraph, for Lack of Enablement

For the following reasons, applicants submit that the subject matter of Claims 10, 18, and 43 is fully enabled by the specification.

Claim 10

The Examiner argues that the specification does not provide enablement for any nucleic acid molecule that encodes a recombinant geranyl diphosphate synthase large subunit protein and that hybridizes to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:1 under conditions of 5 X SSC at 65°C for 16 hours followed by one wash in 1 X SSC at 55°C for 30 minutes.

Applicants note that the hybridization and wash conditions recited in Claim 10 are stringent conditions, and many nucleic acid molecules are unable to hybridize to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:1 under these conditions. For example, nucleic acid molecules that are the same length as the

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complement of the nucleic acid molecule set forth in SEQ ID NO:1 must be about 90% identical to the complement of the nucleic acid molecule set forth in SEQ ID NO:1 in order to hybridize thereto under the hybridization and wash conditions set forth in Claim 10. In this regard, the melting temperature of a DNA duplex can be calculated using the following, art-recognized, equation that is set forth, for example, at page 11.46 of Sambrook et al. (*Molecular Cloning, A Laboratory Manual*, 2d ed., 1989) (submitted herewith as Attachment A):

$$Tm = 81.5 + 16.6(Log_{10}[Na^+])+0.41(\text{fraction G+C}) - (600/\text{length}) \qquad \text{Eq. 1}$$

Wherein N is the length (expressed as the number of nucleotides) of the nucleic acid molecule. As set forth at page 11.47 of Sambrook et al. (submitted herewith as Attachment B), the effect of mismatches on the calculated Tm is to reduce the Tm by 1-1.5°C with every 1% decrease in homology.

The Examiner identifies an oligonucleotide extending from nucleotide 205818 to 205835 of Bacteroides thetaiotamicron VPI-5482 that is 100% identical to nucleotides 1-18 of SEQ ID NO:1. The Examiner characterizes this oligonucleotide as being able to hybridize to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:1 under conditions of 5 X SSC at 65°C for 16 hours followed by one wash in 1 X SSC at 55°C for 30 minutes. The Examiner provided the nucleic acid sequence of this oligonucleotide.

Applicants calculated the melting temperature of the duplex formed between the foregoing oligonucleotide and the complement of nucleotides 1-18 of SEQ ID NO:1 to be 48.9°C in 1 X SSC (0.165 M Na^+). Thus, the foregoing oligonucleotide does not hybridize to nucleotides 1-18 of SEQ ID NO:1 under the conditions of 1 X SSC at 55°C recited in Claim 10.

The Examiner argues that undue experimentation would be required to identify those nucleic acid molecules that hybridize to the complement of the nucleic acid molecule of

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SEQ ID NO:1 under the hybridization and wash conditions recited in Claim 10. Applicants note that the specification, together with the state of the art at the priority date of the present application, provides ample guidance for cloning nucleic acid molecules that encode proteins encompassed by Claim 10. Example 6 of the present application discloses nucleic acid hybridization conditions, and hybridization techniques, that can be used to identify nucleic acid molecules that encode geranyl diphosphate synthase large subunit proteins. The hybridization techniques are art-recognized techniques that are disclosed, for example, in the standard molecular biology laboratory manual edited by Sambrook et al. (*Molecular Cloning, A Laboratory Manual*, 2d ed., 1989).

Additionally, Example 1 of the present application provides an assay for prenyltransferase activity (including geranyl diphosphate synthase activity), and a method for identifying the product (such as geranyl diphosphate) of a prenyltransferase (see, instant specification, page 32, lines 33-35, through page 33, lines 1-33).

Thus, one of ordinary skill in the art can use the teachings of the present application to readily identify and isolate nucleic acid molecules that hybridize to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:1 under conditions of 5 X SSC at 65°C for 16 hours followed by one wash in 1 X SSC at 55°C for 30 minutes. One of ordinary skill in the art can further use the teachings of the present application to readily determine whether the isolated nucleic acid molecule encodes a geranyl diphosphate synthase large subunit protein.

Claim 18

The analysis applied to Claim 10 also applies to Claim 18. Claim 18 is directed to an isolated, recombinant geranyl diphosphate synthase protein comprising an isolated, recombinant geranyl diphosphate synthase large subunit protein and an isolated, recombinant geranyl diphosphate synthase small subunit protein, wherein:

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(a) the isolated, recombinant geranyl diphosphate synthase large subunit protein is encoded by a nucleic acid molecule that hybridizes to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:1 under conditions of 5 X SSC at 65°C for 16 hours followed by one wash in 1 X SSC at 55°C for 30 minutes; and

(b) the isolated, recombinant geranyl diphosphate synthase small subunit protein is encoded by a nucleic acid molecule that hybridizes to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:10 under conditions of 5 X SSC at 65°C for 16 hours followed by one wash in 0.5 X SSC at 55°C for 30 minutes.

Applicants note that the hybridization and wash conditions recited in Claim 18 are stringent conditions, and many nucleic acid molecules are unable to hybridize to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:1 or SEQ ID NO:10 under these conditions. For example, nucleic acid molecules that are the same length as the complement of either the nucleic acid molecule set forth in SEQ ID NO:1, or the nucleic acid molecule set forth in SEQ ID NO:10, must be about 90% identical thereto in order to hybridize under the hybridization and wash conditions set forth in Claim 18.

In particular, as discussed more fully, *supra*, in connection with the rejection of Claim 10, the oligonucleotide from Bacteroides thetaiotamicron VPI-5482 does not hybridize to the complement of nucleotides 1-18 of SEQ ID NO:1 under the conditions of 1 X SSC at 55°C recited in Claim 18. Additionally, the Examiner identifies a poly(A) with 20 adenines that is 100% identical to the poly(A) tail of SEQ ID NO:10. The Examiner characterizes this poly(A) as being able to hybridize to the complement of a nucleic acid molecule consisting of the nucleic acid sequence set forth in SEQ ID NO:10 under conditions of 5 X SSC at 65°C for 16 hours followed by one wash in 0.5 X SSC at 55°C for 30 minutes. Applicants used Equation 1 (Eq. 1) to calculate the melting temperature of the duplex formed between the foregoing poly(A) and the complement of the nucleic acid molecule of SEQ ID NO:10 to be 38.6°C in 0.5 X SSC at 55°C (0.0825 M Na^+). Thus, the foregoing poly(A) does not hybridize to the complement of the

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poly(A) tail nucleic acid molecule of SEQ ID NO:10 under the conditions of 0.5 X SSC at 55°C recited in Claim 18.

For the reasons set forth in connection with the analysis of the Examiner's rejection of Claim 10, applicants submit that undue experimentation would not be required to identify those nucleic acid molecules that hybridize to the complement of the nucleic acid molecule of SEQ ID NO:1, or SEQ ID NO:10, under the hybridization and wash conditions recited in Claim 18, and that encode a geranyl diphosphate synthase large subunit or small subunit protein. In brief, the specification, together with the state of the art at the priority date of the present application, provides ample guidance for cloning nucleic acid molecules that encode proteins encompassed by Claim 18. Additionally, Example 1 of the present application provides an assay for prenyltransferase activity (including geranyl diphosphate synthase activity), and a method for identifying the product (such as geranyl diphosphate) of a prenyltransferase (see, instant specification, page 32, lines 33-35, through page 33, lines 1-33).

Claim 43

The Examiner argues that there is no direction or guidance in the specification to show that amino acids 1-40 of SEQ ID NO:2 can perform the same function of SEQ ID NO:2. Applicants submit that there is no requirement that amino acids 1-40 of SEQ ID NO:2 can perform the same function as SEQ ID NO:2. Amino acids 1-40 of SEQ ID NO:2 comprise a transit peptide that directs a protein attached to the peptide to a cellular membrane. In contrast, SEQ ID NO:2, that includes the transit peptide, is a geranyl diphosphate synthase large subunit protein that catalyzes the formation of geranyl diphosphate synthase.

Rejection of Claim 43 Under 35 U.S.C. § 112, First Paragraph, for Lack of Written Description

An applicant shows possession of the claimed invention by describing the claimed invention with all of its limitations using such descriptive means as words, structures, figures, diagrams, and formulas that fully set forth the claimed invention. (M.P.E.P., Rev. 2, May 2004,

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section 2163, citing *Lockwood v. American Airlines, Inc.*, 41 U.S.P.Q.2d 1961, 1966 (Fed. Cir., 1997)).

Claim 43 is directed to an "isolated recombinant geranyl diphosphate synthase large subunit protein of Claim 10 comprising a transit peptide consisting of amino acids 1-40 of SEQ ID NO:2." Applicants submit that the instant application clearly discloses and claims SEQ ID NO:2, which is an example of an isolated recombinant geranyl diphosphate synthase large subunit protein of Claim 10 comprising a transit peptide consisting of amino acids 1-40 of SEQ ID NO:2. One of ordinary skill in the art would readily recognize that applicant is in possession of the subject matter of Claim 43, and that the subject matter of Claim 43 is part of the invention. Thus, applicants submit that the subject matter of Claim 43 is described by the instant specification.

CONCLUSION

In view of the foregoing arguments, applicants submit that all of the claims are in condition for allowance. Reconsideration and favorable action are requested.

Respectfully submitted,

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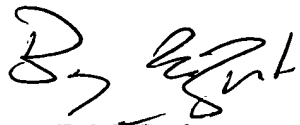

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CALCULATING MELTING TEMPERATURES FOR PERFECTLY MATCHED HYBRIDS BETWEEN OLIGONUCLEOTIDES AND THEIR TARGET SEQUENCES

When using single oligonucleotides that match the target sequence perfectly, hybridization conditions can easily be derived from the calculated T_m of the hybrid. For oligonucleotides shorter than 18 nucleotides, the T_m of the hybrid can be estimated by multiplying the number of A + T residues in the hybrid by 2°C and the number of G + C residues by 4°C and adding the two numbers (Itakura et al. 1984). However, this method overestimates the T_m of hybrids involving longer oligonucleotides.

A different approach has been taken by E. Fritsch (unpubl.), who found that the equation originally used to calculate the relationship between G + C content, ionic strength of the hybridization solution, and the T_m of long DNA molecules (Bolton and McCarthy 1962):

$$T_m = 81.5 + 16.6(\log_{10}[Na^+]) + 0.41(\text{fraction G + C}) - (600/N),$$

where N = chain length, predicts reasonably well the T_m for oligonucleotides as long as 60–70 nucleotides and as short as 14 nucleotides.

This formula only works for Na^+ concentrations of 1 M or less.

ESTIMATING THE EFFECTS OF MISMATCHES

Perhaps surprisingly, the classic formula (Bonner et al. 1973) to calculate the effect of mismatches on the stability of long DNA hybrids holds reasonably well for hybrids involving short oligonucleotides: For every 1% of mismatching of bases in a double-stranded DNA, there is a reduction of T_m by 1–1.5°C. However, the precise effect of mismatches depends on the G + C content of the oligonucleotide and, even more critically, on the distribution of mismatched bases in the double-stranded DNA. Mismatches in the middle of the oligonucleotide are far more deleterious than mismatches at the ends. Therefore, the method of estimation given above should only be used as a rough guide until a systematic study of all types of mismatches in a variety of contexts leads to more precise methods of estimation. If appropriate target DNA has been cloned, the effect of mismatches on T_m can be determined empirically (see pages 11.55–11.57).